PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF *PIPER RETROFRACTUM* VAHL. AND *PIPER ARBORESCENS* ROXB.

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For my beloved father, mother, sister and grandmother

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ABSTRACT

The phytochemical investigations of the fruits of *Piper retrofractum* Vahl. and the stems and leaves of *Piper arborescens* Roxb. have been carried out. The fruits of *Piper retrofractum* Vahl. were bought from a spice shop at Pandan, Johor Bahru and the methanolic crude extracts of stems and leaves of *Piper arborescens* Roxb. were obtained from UiTM Sarawak, Malaysia. Samples were extracted with Soxhlet extractor using methanol as the solvent to give the crude extracts. The crude extracts were fractionated using vacuum-liquid chromatography and then purified using several chromatographic techniques. The isolated compounds were identified by analysis of various spectral data using infrared, 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, and HMBC) nuclear magnetic resonance spectroscopies and mass spectrometry. Seven compounds were isolated from these plants. The purification of methanol crude extract of the fruits of *Piper retrofractum* Vahl. yielded four compounds identified as piperine, oleic acid, *N*-isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide and methyl piperate. Another three compounds, characterized as sesartemin, diayangambin and 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol had been isolated from the methanol crude extract of the stems of *Piper arborescens* Roxb. The hydrolysis of piperine was done using potassium hydroxide and ethanol to yield piperic acid. The antibacterial test against Gram positive bacteria (*Bascillus subtilis* and *Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were performed on the crude extracts and isolated compounds. The crude extracts and piperine were found to show the strongest inhibition against both Gram positive bacteria with MIC and MBC values of 225 µg/mL.

ABSTRAK

Kajian fitokimia ke atas buah *Piper retrofractum* Vahl., batang dan daun *Piper arborescens* Roxb. telah dijalankan. Buah *Piper retrofractum* Vahl. dibeli daripada kedai rempah di Pandan, Johor Bahru dan ekstrak mentah metanol daripada batang dan daun *Piper arborescens* Roxb. diekstrak di UiTM Sarawak, Malaysia. Sampel telah diekstrak menggunakan Soxhlet dengan metanol sebagai pelarut untuk mendapatkan ekstrak mentah. Ekstrak mentah diperingkatkan menggunakan kromatografi cecair vakum dan kemudian ditulenkan dengan menggunakan beberapa teknik kromatografi. Sebatian tulen dikenalpasti dengan analisis data pelbagai spektrum iaitu spektroskopi inframerah, 1D (^{1}H, ^{13}C dan DEPT) dan 2D (COSY, dan HMBC) resonans magnet nukleus dan spektrometri jisim. Tujuh sebatian telah dipisahkan daripada tumbuhan kajian ini. Penulenan ekstrak mentah metanol buah *Piper retrofractum* Vahl. menghasilkan empat sebatian tulen yang dikenalpasti sebagai piperina, asid oleik, *N*-isobutil-2*E*,4*E*,14*Z*-eikosatrienamida dan metil piperat. Tiga sebatian yang dicirikan sebagai sesartemin, diayangambin dan 3-(3,4-dimetoksihidroksibenzil)-4-(3',4',5'-trimetoksibenzil)-tetrahidrofuran-2-ol telah dipisahkan daripada ekstrak mentah metanol bahagian batang *Piper arborescens* Roxb. Hidrolisis piperina dilakukan dengan menggunakan kalium hidroksida dan etanol untuk menghasilkan asid piperik. Ujian antibakteria terhadap bakteria Gram positif (*Bascillus subtilis* dan *Staphylococcus aureus*) dan bakteria Gram negatif (*Pseudomonas aeruginosa* dan *Escherichia coli*) telah dilakukan ke atas ekstrak mentah dan sebatian tulen. Ekstrak mentah dan piperina didapati menunjukkan perencatan terkuat terhadap kedua-dua bakteria Gram positif dengan nilai MIC dan MBC 225 µg/mL.